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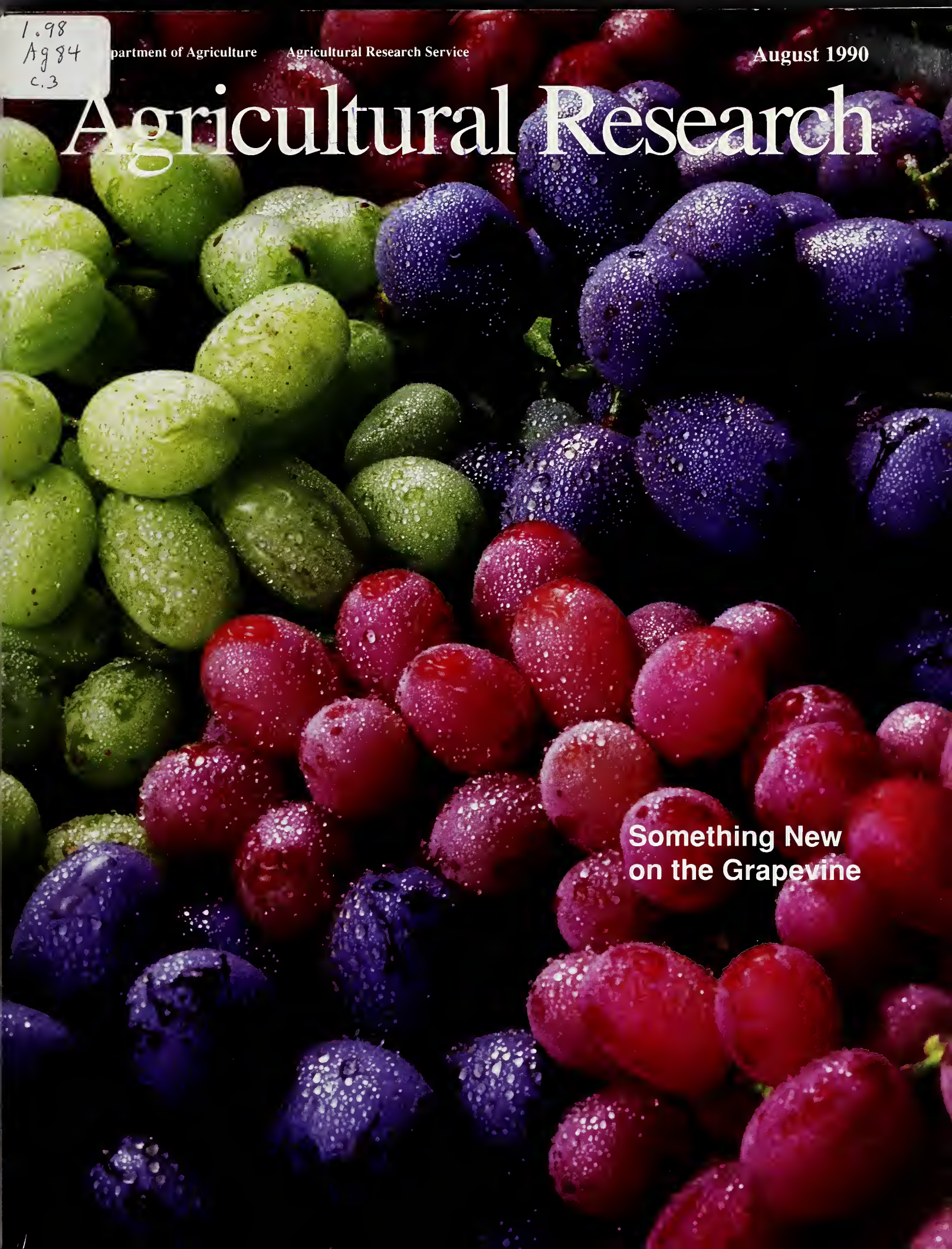

Something New on the Grapevine

The Good, the Sweet, and the Seedless

"When they're good, they're soooo good, I can't stop eating them," announced one of our coworkers, popping a grape into her mouth. "But sometimes I buy them, and they're sour. What a disappointment!"

It's an old story. Even in Aesop's day, sour grapes were notorious for tantalizing the eye yet offending the tongue. So how's a modern-day grape buyer to spot a fresh, sweet bunch of grapes, even when distanced from them by peekaboo plastic wrap?

Things were easier in grandmother's day, when helping oneself to a sample was a common practice at the corner grocery. Today, grocers discourage sampling for several reasons: unauthorized food handling often proves unsanitary. Then too, missing berries hasten spoilage and degrade the value of remaining fruit clusters—a hidden cost that may be passed on to customers.

Of course, the matter of ripeness begins in the vineyard. Sour grapes are typically grapes that have been picked before or at the stage when berries begin to soften and color. Ripening, according to "Grape Growing," by Robert J. Weaver, "consists mainly of an increase in sugar, a decrease in acidity, and the development of a characteristic color, texture, and flavor. These changes occur only as long as the grapes remain on the vine and practically cease after picking."

A significant milestone in the quest for grandeur on the grapevine was attained in the mid-60's, although few realized it at the time. That's when Agricultural Research Service horticulturist John H. Weinberger began his work on Flame Seedless, a cross that incorporated five other varieties.

Although Flame was released by USDA in 1973, it took at least 3 years for commercial vines to bloom, and another few for production to peak. Consequently, nearly a decade elapsed before the new variety fulfilled its commercial potential and found its niche at the supermarket.

But when Flame caught fire... Wow! Consumers purchased 250,000 boxes in 1980; 3 years later, more than 3 million boxes were sold. Today, it's America's best-selling red seedless grape.

Flame sports a ripeness advantage that pickers have learned to appreciate: When it turns red, it's ripe—and can be trusted to be fully sweet. The grape-gobbling public knows that Flame will not disappoint.

Wholesalers like it too. According to a 1984 article in *Fortune*, Flame is firmer than the original Thompson seedless grape; therefore, it travels well and keeps longer in cold storage.

ARS viticulturists are understandably fond of their old Flame but admit that new, ever-more-promising varieties—Fantasy Seedless, Crimson Seedless—also tug at their heartstrings. Recent improvements in the technology of grape breeding, whether conventional or with genetic engineering, have vastly eased and accelerated the processes by which new vines are cultured. "Grape Research Presses On," page 4, by Marcia Wood has the juicy details.

And is the public ready for the new varieties to come to market? Probably so if recent years are any indication. Per capita consumption of fresh grapes increased from 3.09 pounds in 1978 to 8.03 in 1988, up 260 percent.

Grape growers doubled their production during the same period, with much of the additional grapes coming from seedless varieties.

During the same time, imports of grapes, mostly from Chile, increased 6-fold, a fact that is good news for grocery shoppers, and not necessarily bad news for American producers. Coming in our off-season as they do, grapes imported from South America generally don't compete in the marketplace with domestic ones, and they tend to keep consumers thinking fresh table grapes at a time when U.S.-grown produce is not available.—By **Regina Wiggen,** Associate Editor

Agricultural Research

Cover: Better, sweeter, juicier grapes—that's the goal. Four new ARS varieties are Crimson Seedless (red), Autumn Black (upper right), Fantasy Seedless (blue-black), and Autumn Seedless (green). Photo by Jack Dykinga. (K-3682-9)

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JACK DYKINGA

Grape Researchers Press Ahead

Sweet, juicy grapes—picked at the peak of ripeness—are one of nature's best-tasting treats. In vineyards, greenhouses, and laboratories, ARS scientists seek even better grapes for tomorrow.

The ARS laboratory that developed America's most popular red seedless grape, Flame Seedless, more recently offered nurseries and breeders a delicious new black seedless grape for midsummer. Called Fantasy Seedless, the newcomer is a sweet, firm, fresh-market grape.

ARS horticulturist David W. Ramming at Fresno, California, and his team developed the new grape, and earlier varieties such as Autumn Black and Autumn Seedless. "Because Fantasy's berries are naturally large Ramming says, growers don't need to boost

Technician Ronald Tarailo examines raisin-grapes being dried in the traditional manner near Fresno, California. (K-3683-10)

berry size by applying gibberellic acid or girdling the plants."

Grapegrowers routinely spray gibberellic acid, a plant hormone, on almost all seedless grape vineyards, to enhance berry growth. Some seedless vines also require girdling—in which a narrow, shallow incision around the vine's trunk temporarily interrupts the normal flow of nutrients—to the berries' advantage.

Every major grape nursery in California, the leading producer of fresh-market grapes, has asked for cuttings of Fantasy, as well as of another recent offering from the Fresno lab—a bright red grape named Crimson Seedless. Intended for September and October harvest, the new grape may eventually replace Emperor, a seeded red grape that ripens at about the same time.

Seedlessness gives Crimson a competitive advantage over Emperor. "People don't like to spit out seeds," says Ramming.

Fantasy Seedless and Crimson Seedless could show up in supermarket produce sections in 3 to 5 years. Both were the product of conventional breeding techniques; that is, a seeded and a seedless parent were crossed, yielding brand new, experimental hybrids.

But the researchers also use a sophisticated laboratory technique called embryo rescue, which they pioneered for grape breeding.

Both approaches may help the scientists tackle many remaining challenges. An example: They want to offer a tasty white (what shoppers consider green) seedless grape that's ready in May, like the popular Perlette variety. Unlike Perlette, however, the hoped-for white seedless of tomorrow wouldn't require costly hand-thinning.

And embryo rescue may someday yield a new seedless muscat-flavored grape that might prove ideal for fresh-market (table grape) sales or perhaps for raisinmaking. "There's a muscat table grape called Italia already on the market," says Ramming, "but it's seeded.

"Muscat of Alexandria, used for drying, also has seeds. That means raisin packers have to punch the seed out, breaking the skin and making the muscat raisins stick together inside the box."

Popular in Europe, muscats have a strong, aromatic flavor. If you've ever eaten chocolate-covered raisins, you may have tasted muscats.

JACK DYKINGA

Immature seeds— too small for most people to detect—are removed from seedless grapes for study. (K-3684-5)

The Fresno team also wants to offer tomorrow's growers a raisin grape that boasts all of the time-tested qualities of Thompson seedless—mainstay of California's $600-million-a-year raisin industry—yet is ready to harvest at least 10 to 21 days earlier. As things stand now, grapes drying on paper trays on the vineyard floor occasionally get hit by unseasonably early rains, which can hasten rot.

Top contenders right now are seven experimental types of raisin grapes that bear fruit about 14 days sooner than Thompson. But the ultimate raisin grape would dry naturally on the vine so that it could be quickly harvested by fast-moving, mechanized shakers, instead of by the hand labor that's expected to become increasingly scarce.

"If you leave Thompson seedless or Fiesta raisin grapes on the vine, they'll just rot," says research technician Ronald E. Tarailo at Fresno. But the dozen or so dried-on-the-vine types Tarailo has happened on in experimental vineyards "shrivel and turn into raisins right on the vine."

Although these vines look promising, those that don't measure up are chopped down and uprooted to make way for new contenders.

This past spring, for example, the Fresno team handplanted almost 10,000 new hopefuls. Some of those raisin grapevines are the product of conventional breeding. But others got their start in life through the embryo rescue process.

Ramming and ARS colleague Richard L. Emershad, a plant physiologist at Fresno, adapted embryo rescue from earlier work done with cotton. The technique accelerates breeding of seedless grapes by permitting the successful cross of one seedless parent with another to produce a healthy seedling. That feat is not normally possible in nature, or with conventional breeding.

More than half of the vines grown from the embryo-rescue seedlings bear seedless fruit. In contrast, more than 70 percent of the seedlings from conventional breeding's option—the crossing of seeded to a seedless parent—yield seeded grapes, useless in raisinmaking and equally undesirable for the fresh-grape market.

So-called seedless grapes have seeds inside, but they're immature and too small for most people to detect. In nature, such seeds usually abort when the grape forms, so they

JACK DYKINGA

Graduate student Lidia Lee and plant physiologist Richard Emershad transplant seedless grape varieties from growth chamber containers to soil pots for the greenhouse. (K-3681-7)

can't be used to produce new vines of seedless grapes.

With embryo rescue, however, scientists cross one seedless parent vine with another, wait for the pollinated flowers to form grapes, and then carefully remove immature seed from these berries. If nurtured on special nutrients in the laboratory, rescued embryos may develop into seedlings that scientists will later move to the greenhouse and finally to experimental vineyards for testing.

Shorter Quarantine

To build better grapes for tomorrow, breeders like Ramming need ready access to vines or cuttings of important new grapes from around the world. Those grapes might boast colors or flavors not offered in today's U.S.-grown varieties, or might provide enhanced resistance to grapevine insect or disease pests.

Cornell University's Dennis Gonsalves at Geneva, New York, and ARS' Deborah A. Golino at Davis, California, want to cut short the time imported vines spend in quarantine, awaiting a clean bill of health.

"Because the diseases and pests that imported grapevines might carry could pose a serious threat to American vineyards," says Golino, "grapevines aren't allowed into the United States except under carefully controlled quarantine conditions." Quarantine prevents the accidental introduction of virus or viruslike diseases such as grapevine leafroll or corky bark.

Grapevine leafroll virus disease could spread if imported cuttings from infected mother vines were grafted to rootstocks that were then planted in U.S. vineyards.

To prevent that from happening, pieces of the imported vine are grafted to indicator grapevines, highly vulnerable to these and other diseases. The grafted plant then spends 2 years in quarantine—long enough to spot any symptoms.

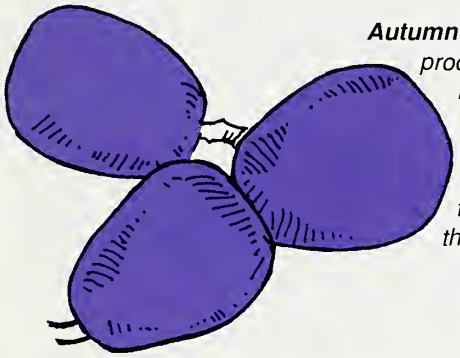
In research financed by ARS' gene bank for grapes and other crops in Geneva, New York, Gonsalves devised biotechnological tests (enzyme linked immunosorbent assays, or ELISA's) for detecting three of the four known strains of grapevine leafroll virus disease. Leafroll, so named because leaves of diseased plants roll downward, causes yield losses of 20 percent each year on infected vines.

The new assay yields, in a matter of days, results that would otherwise take years to obtain from the indicator-grapevine test.

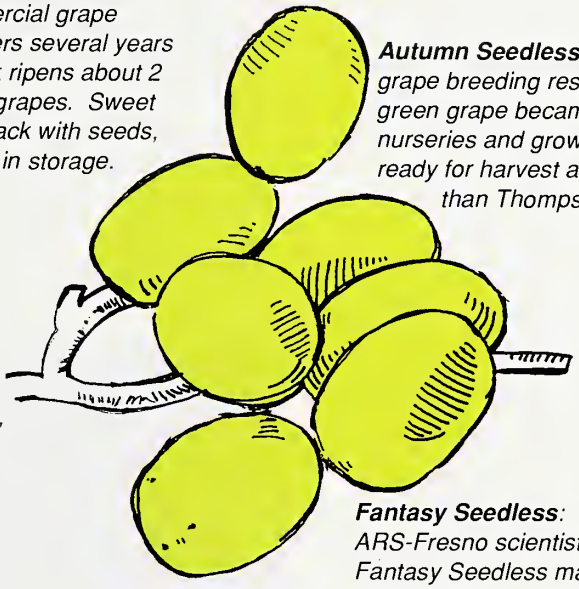
Another equally rapid tactic for finding the virus requires isolating its genetic material (double-stranded RNA, or dsRNA) from grapevine tissue. By the end of this year, Gonsalves hopes to have results from concurrent comparisons of the

(Continued on page 8)

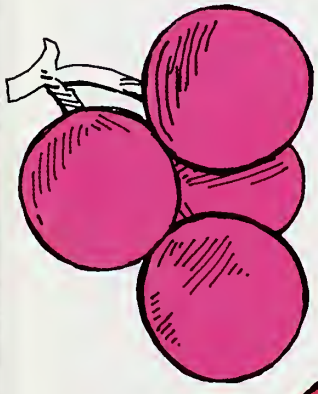
The Grapes of Craft



Autumn Black: *Now in small-scale production after its ARS developers released it to commercial grape growers and breeders several years ago, Autumn Black ripens about 2 weeks after Ribier grapes. Sweet tasting and bluish-black with seeds, this fruit holds up well in storage.*

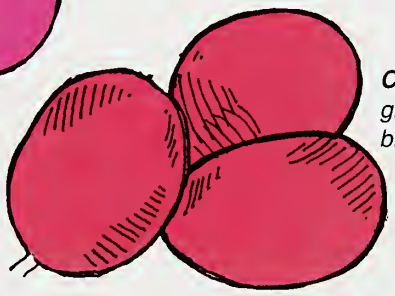


Autumn Seedless: *A product of ARS grape breeding research, this light-green grape became available to nurseries and growers in 1984. It is ready for harvest about 2 weeks later than Thompson seedless.*



Flame Seedless*: Released in 1973, its firm, crisp texture and sweet taste have made it a produce section favorite. It is the best known of the fresh-market grapes originating from ARS grape breeding research at Fresno, California.*

Fantasy Seedless*: Released by ARS-Fresno scientists in 1989, Fantasy Seedless may show up in supermarket produce sections within the next 3 to 5 years.*



Crimson Seedless*: This bright red grape was distributed to grape breeders in 1989 by ARS-Fresno scientists. If it meets breeders' exacting requirements, Crimson Seedless could arrive on the market as early as 1993.*

Improving on Nature's Packaging

With just the right packaging, at least one new seedless grape variety could aid midwestern growers trying to compete in the table grape market.

Venus, Reliance, and Saturn, all varieties from the Arkansas Agricultural Experiment Station breeding program, can be held at 37.4°F for 4 weeks before any apparent quality loss. Beyond that, how well the grapes survive depends greatly on how the fruit is packaged.

According to Penelope Perkins-Veazie, postharvest physiologist at ARS' South Central Agricultural Research Laboratory in Lane, Oklahoma, research examined three factors in packaging and storage: disease, total weight loss, and shatter—the tendency to fall from the cluster as they ripen.

Results showed that Saturn, after 6 weeks of storage, fared the best of the three varieties when packaged in cardboard boxes covered with shrink-wrap. This variety demonstrated the greatest potential for long-distance shipment and storage without sulfur dioxide, used to control mold. Venus rated the worst, shattering after 4 weeks and suffering significant weight loss at 5.

Boxes with shrink-wrap covering provided the most insurance against loss, followed by plastic dome-lid boxes with vent holes. Net bags, often used for California table grapes at retail markets, proved poor safeguards for all varieties, particularly Venus.—By **Matt Bosisio,** ARS.

Penelope Perkins-Veazie is at the USDA-ARS South Central Agricultural Research Laboratory, P.O. Box 159, Lane, OK 74555 (405) 889-7395.

(Continued from page 6)
conventional quarantine test and the newer, speedier assays.

Gonsalves may be the first to produce useful molecules (antisera) that signal the presence of another grapevine enemy, the viruslike corky bark disease.

Diseased vines develop unnaturally thickened bark and pinkish inner wood. And although corky bark seldom kills a plant outright, it has "subtle, pervasive effects," says colleague Golino. A vine, once infected, will "never lose the corky bark microorganism," she says.

Messing Up Insect Mating

While diseases are challenging these scientists, other researchers are trying to outwit the worst insect enemy of southern U.S. vineyards—the grape root borer. A pheromone-based lure, targeted at the borer's clearwing moth stage, disrupts mating and thus helps protect the crop—without insecticides, says entomologist J. Wendell Snow, formerly at Byron, Georgia and now at Honolulu, Hawaii.

Two of the borer's fat, wormlike larvae can easily kill a young vine, says Snow, who helped increase potency of the ARS-developed lure. Preliminary tests indicate that 100 pheromone-filled plastic strips apparently provide up to 80 percent control of the grape root borer in an acre of vineyard.

Harnessing Soil Fungi

Meanwhile, in California's Napa Valley, newly planted grapevines might get off to a better start if beneficial soil microorganisms called mycorrhizal fungi are within easy reach of emerging roots, says research leader Robert G. Linderman at Corvallis, Oregon. He's asked viticulturists from some of Napa Valley's best known wineries to dip shoestringlike roots of some 15,000 vines into a gel that carries a hormone and tiny clay chips containing the live microorganisms.

Linderman expects the fungi to enhance plant growth by helping vines escape transplant shock and other stresses. "The fungal strands that grow from the mycorrhizae will be especially effective in piping phosphorus to plant roots," he says. In many soils, including those of the Napa Valley, phosphorus is normally tightly bound to clay particles and difficult for unaided roots to capture.

JACK DYKINGA

Technician Sherilyn Boynton examines a tissue-cultured grape plantlet bred from seedless crosses. (K-3683-13)

Weed Control Without Groundwater Worries

In the race to seize essential nutrients from soil, vines can lose out to weeds such as barnyardgrass, green foxtail, lambsquarters, and pigweed. But slow, controlled release of herbicides, encapsulated in ARS-developed cornstarch granules, might heighten herbicide effectiveness yet reduce leaching of the chemical into groundwater, says plant physiologist Rick A. Boydston.

With luck, findings from his experiments with drip-irrigated or sprinklered plots in a Prosser, Washington, vineyard will validate his indoor tests. In those, encapsulated herbicide "largely stayed in the top half-inch to 1 inch of soil, where it was most likely to come in contact with sensitive roots of weed seedlings." In a conventional formulation, herbicide traveled 5 to 6 inches down into the soil.

Precision irrigation is one way to help stop leaching of nitrate fertilizer into groundwater, says Claude J. Phené, soil scientist at Fresno. Phené, colleague Larry E. Williams of the University of California at Davis, and others are fine-tuning irrigation requirements of young vineyards that are watered with surface or subsurface drip systems.

Using less water than many California grape growers apply, the scientists reaped an impressive 14.7-ton-per-acre harvest from 2-year-old Thompson seedless vines, a variety that typically yields 9 to 12 tons per acre, beginning at age 3.

When grapes such as Thompson Seedless are put in cold storage, repeated fumigation with sulfur dioxide protects the harvest from *Botrytis* rot, a gray mold that thrives in the cold. To help cold storage houses stay under the 10-part-per-million limit for sulfite residues in grapes, plant pathologist Joseph L. Smilanick and biological technician Delmer J. Henson at Fresno, along with university researchers, are scrutinizing every step of the conventional fumigation process.

California's Table Grape Commission is funding the research.

By checking grape pH and temperature, airflow, humidity, and pallet-stacking patterns, researchers hope to provide new tips for getting maximum results from minimum applications of the gas. Payoffs should include increased safety for consumers who are allergic to sulfites, plus reduced emission of the fumigant, an air pollutant.—By **Marcia Wood,** ARS.

[If you are interested in contacting scientists mentioned in this article, write or telephone the Editor, Agricultural Research, Bldg. 005, Beltsville Agricultural Research Center-West, Beltsville, MD 20705 (301) 344-3280.] ◆

JACK DYKINGA

Dried-on-the-vine grapes, here checked by technician Ronald Tarailo, might make mechanical raisin harvesting feasible. (K-3683-3)

Reviving an American Tradition

Prohibition and the Great Depression of the 30's virtually wiped out the wine industry based on a native American grape, the muscadine. ARS scientists are now helping the muscadine make a comeback—not as wine—but as a juice beverage.

"We've developed new planting and growing practices that increase yield and fruit quality with fewer chemicals and less labor," says horticulturist James M. Spiers at the ARS Poplarville, Mississippi, Small Fruits Research Station. Spiers has been working with the bronze or purple-black fruit since 1987.

Although the vines grow vigorously when mature, he says it's difficult to establish them initially. William C. Olien (formerly at Poplarville; now with Clemson University) found that planting vines in larger holes and avoiding a smooth or polished soil surface around the hole sides increases plant survival rate.

"Conventional planting in small, auger-drilled holes creates a highly polished soil surface that restricts root growth," Olien explains.

Olien also found that adding peat to the soil helps maintain moisture and nutrients and minimum pruning improves growth and development.

"These new practices could produce a commercial crop in the third year," Spiers says. It can take up to 5 years using current methods.

ARS has been working with the Agriculture and Forestry Experiment Stations at Starkville, Beaumont, and Crystal Springs, Mississippi, to revive the muscadine industry.

A major muscadine juice and processing plant was just completed in Ellisville, Mississippi, with a satellite plant in Rose Hill, North Carolina.

"The juice, which tastes light and bubbly like champagne, is quite popular in the Southeast. We're looking forward to an expanding market," Spiers says.—By **Doris Sanchez,** ARS.

DON ALBERN

Curator Philip Forsline checks the heavy fruiting characteristics of a French-American grape hybrid at the Geneva, New York, clonal germplasm repository. (K-2556-4)

Gene Banks: Treasure Trove of Grape Varieties

America's grape gene banks—in Geneva, New York, and Davis, California—safeguard vines of wild and cultivated grape varieties from around the world.

The Geneva collection emphasizes cold-hardy grapes that can withstand the below-freezing temperatures of some U.S. grape-growing regions, including upstate New York, the repository's home, says curator Philip L. Forsline. The collection boasts also grape varieties from China and Russia and the best U.S. assemblage of native American grapes.

Studies funded by the Geneva repository include collaboration with Colorado State University researcher Cecil Stushnoff, who's seeing if buds snipped from dormant vines can be safely plopped into cryopreservation tanks filled with liquid nitrogen, a common practice for successfully storing many types of valuable seeds. If it works, this space-saving tactic would make room at Geneva and Davis for more of the world's grape varieties now threatened by industrialization or natural hazards such as insects, disease, or drought.

Like all other imported grapevines, those headed for the Geneva or Davis collections have to go through lengthy quarantine to ensure they're insect- and disease-free. Curator Kathleen S. Rigert at Davis says cutting the time it takes to graft pieces of incoming material to indicator grapevines—one step in disease testing—might shorten quarantine delays.

Rigert and Deborah A. Golino, an ARS plant pathologist at Davis, are comparing three types of grafting. "We want to see if our chances of detecting infections of leafroll, corky bark, or *Rupestris* stem pitting are just as good with one type of graft as another," says Rigert.

The grape collection at Davis features varieties suited for western U.S. vineyards. The California bank also houses an outstanding assortment of unusual grapes from the Middle East, Eastern Europe, and the warmer regions of Russia, and the world's most extensive selection of wild relatives of cultivated grapes.

Destined for the Davis gene bank are findings from the 1988 Far Eastern journey of plant explorer Maxine M. Thompson, emeritus professor at Oregon State University. In remote mountain valleys of Pakistan where the Himalaya, Karakorum, and Hindu-Kush ranges meet, Thompson gathered more than five dozen grapevines—each unique. One is a wild grape that "doesn't seem to get diseased and looks particularly interesting for our humid southeastern states," she says.

More recently, forays by M. Andrew Walker, assistant professor at University of California at Davis, into dry ravines and brushy scrublands of Texas yielded wild grapes that flourish in droughty climes where other vines might wither and die. Walker's botanical loot, now at Davis, may harbor valuable genes for drought tolerance. That would be a boon to future generations of California grapevines, grown in regions where competition for water will surely become increasingly fierce.—By **Marcia Wood,** ARS.

Grass-Fungus Symbiosis Saving Lawns

Homeowners may find it a cinch to foil the hairy chinch bug and several other lawn pests simply by ordering fungus with their grass seed.

Fungal endophytes—fungi that live within a plant but do no damage—have been found to confer exceptional resistance to the hairy chinch bug on turf grasses such as perennial ryegrass and tall fescue.

Hairy chinch bugs ("chinche" is Spanish for bug) are sporadic but voracious destroyers of grass that can severely damage large areas of lawn almost overnight. Chemical treatments to control them are expensive and may harm beneficial insects.

"The insects just don't feed on grasses that have high levels of the endophyte when they have any other choice," says ARS researcher Roger H. Ratcliffe.

Fungal endophyte infection has been associated with insect resistance and increased vigor and drought tolerance in the past, but Ratcliffe and his team were able recently to measure the degree of protection provided by the fungi against the hairy chinch bug. Ratcliffe is an entomologist at ARS' Beltsville (Maryland) Agricultural Research Center.

"When chinch bug nymphs fed on test plots of 14 different perennial ryegrasses, those grasses that were more than 50 percent infected by the endophyte scored in the top half of appearance," Ratcliffe says. "Others with low endophyte populations had large spots where the grass was killed."

The endophyte produces alkaloid compounds that grass-feeding insects such as the hairy chinch bug, the bluegrass webworm, and others avoid. "They just go elsewhere if it's at all possible," Ratcliffe says.

In tests where the hairy chinch bug was given no other choice but to live off endophyte-infected ryegrass, only 4 percent of the adult bugs survived compared to a 93-percent survival rate on uninfected ryegrass. "They seem to starve to death. They may also be killed by toxins in the grass," he says.

Grass seed must be infected before it is planted, since the endophyte cannot spread from plant to plant, Ratcliffe points out.

Several grass varieties are already being marketed as endophyte-infected. "But seed must be stored properly for the endophyte to remain in good condition," Ratcliffe says. "After 12 to 18 months of storage, the level of viable endophyte drops off rapidly if the grass seed has been stored at a high temperature."

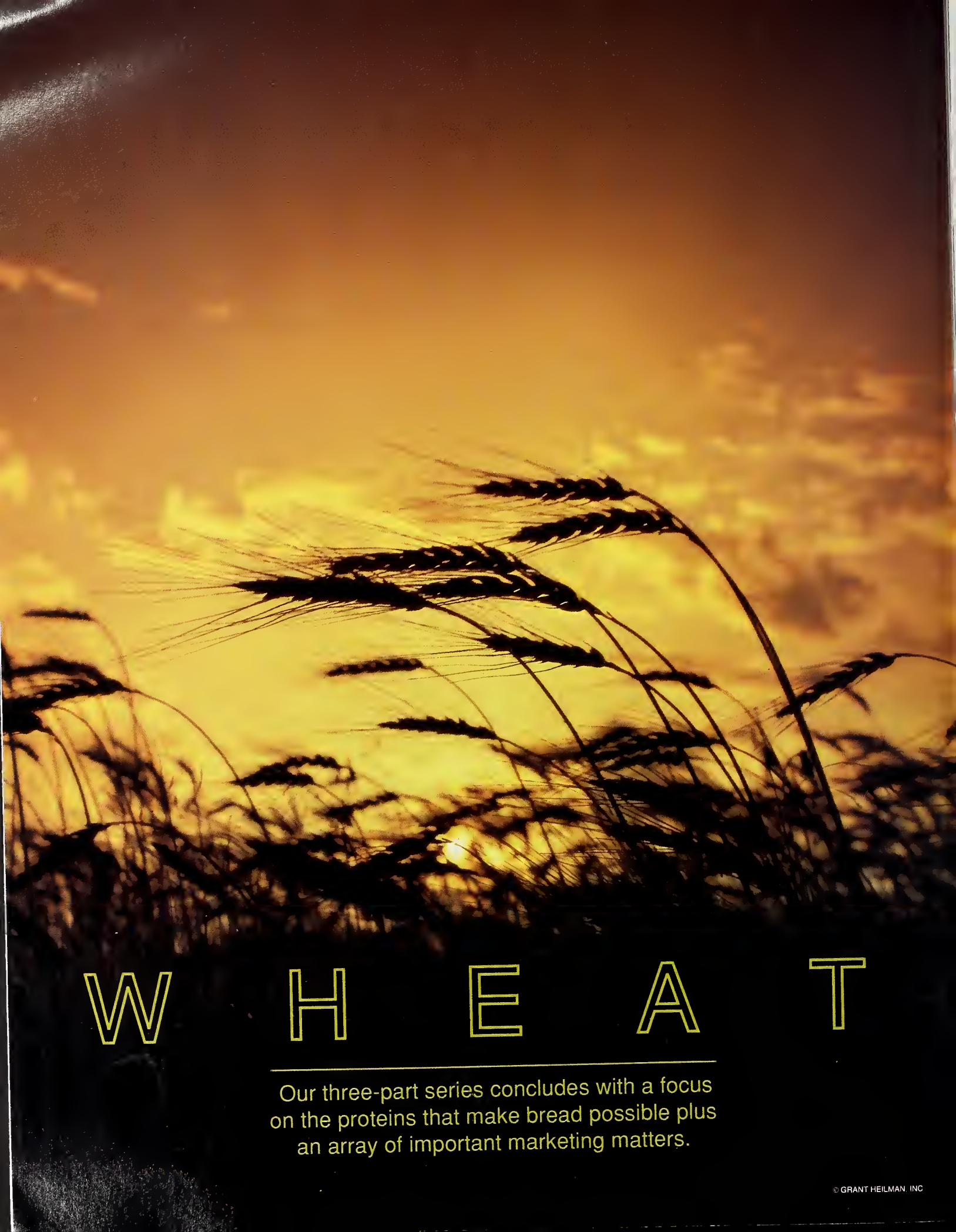
As Ratcliffe and other researchers work out the mechanism of resistance, they are also starting to consider looking for endophytes that will do the same type of job in other turf grasses. "We're pretty sure there are species-specific endophytes for many of the cool season turf grasses and possibly some of the warm season ones as well," Ratcliffe says.

"We could go a long way toward low maintenance lawns and less chemical use if we can put endophytes to work," he adds.—By **J. Kim Kaplan,** ARS.

Roger H. Ratcliffe is at the USDA-ARS Soybean and Alfalfa Research Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705 (301) 344-2392. ◆

KEVIN MORRIS

A harmless internal fungus protected perennial ryegrass on right of photo from damage by the hairy chinch bug.



W H E A T

Our three-part series concludes with a focus on the proteins that make bread possible plus an array of important marketing matters.

Breathing New Life Into an Old Crop

Fresh, fragrant bread from America's commercial bakeries could be even better, bakers say, if wheat flours were of better quality.

For a baker, the best flours make dough that is strong, stretchy, highly elastic, and yet easy to work with. Finished loaves from such doughs are high, full, light, and uniform, with just the right crumb texture.

Across the country, ARS scientists who work with wheat aim to make U.S.-grown grain consistently meet the needs of producers, marketers, and consumers—here and abroad.

Sleuthing Secrets of Wheat Glutens

Wheat is the only grain that can be made into a dough that rises and produces light, fluffy baked goods. And wheat's most important doughmaking components are its gluten proteins. Glutens enable wheat flours to produce strong yet pliable doughs.

Naturally enough, gluten has captured the interest of plant breeders and genetic engineers trying to tailor the protein for a wide array of foods. But learning how gluten fits together and interacts with other food components such as starch and lipids in bread may be among the most challenging problems faced by protein chemists, says ARS chemist Jerold A. Bietz at Peoria, Illinois.

Gluten has some 100 to 200 different subunits joined in "an incredible number of combinations that may affect the proteins' nutritional and physical qualities," Bietz says. He leads ARS' plant protein research at the Northern Regional Research Center.

During the past 30 years, milestones achieved by Center scientists have helped unlock some of gluten's secrets. For example, one Peoria research team has discovered better ways to isolate gluten's two main constituents—gliadins and glutenins.

Chemist Floyd R. Huebner at Peoria is helping wheat breeders identify gliadins that relate to baking quality. He uses a high-tech laboratory procedure, reversed-phase high performance liquid chromatography (RP-HPLC). Huebner studies wheats from ARS' Hard Red Spring and Durum Wheat Quality Laboratory, Fargo, North Dakota.

The Peoria scientists are trying to make it easier for geneticists to select high-quality wheat types earlier in the breeding process. This is possible because samples as small as half a kernel may be analyzed. Other approaches typically require a large quantity of uniform seed, which may take years to produce. "There's an incredible amount of information available through this type of liquid chromatography," Huebner says.

Protein Declines, Researchers Fight Back

Protein content of hard red winter wheat in Nebraska is declining, and the crop's milling and baking properties are suffering as a result. ARS

plant geneticist Robert A. Graybosch and coworkers at the University of Nebraska, Lincoln, are among the scientists trying to discontinue the unwanted trend.

Hard red winter wheat is the major cash crop for farmers in the western half of the Great Plains.

Wheat breeding calls for wheat lines that are not only high in protein, but also resistant to disease and insects. "In striving for high yields and insect and disease resistance, breeders may have inadvertently brought about the decrease in protein that we see today," says ARS agronomist C. James Peterson at Lincoln.

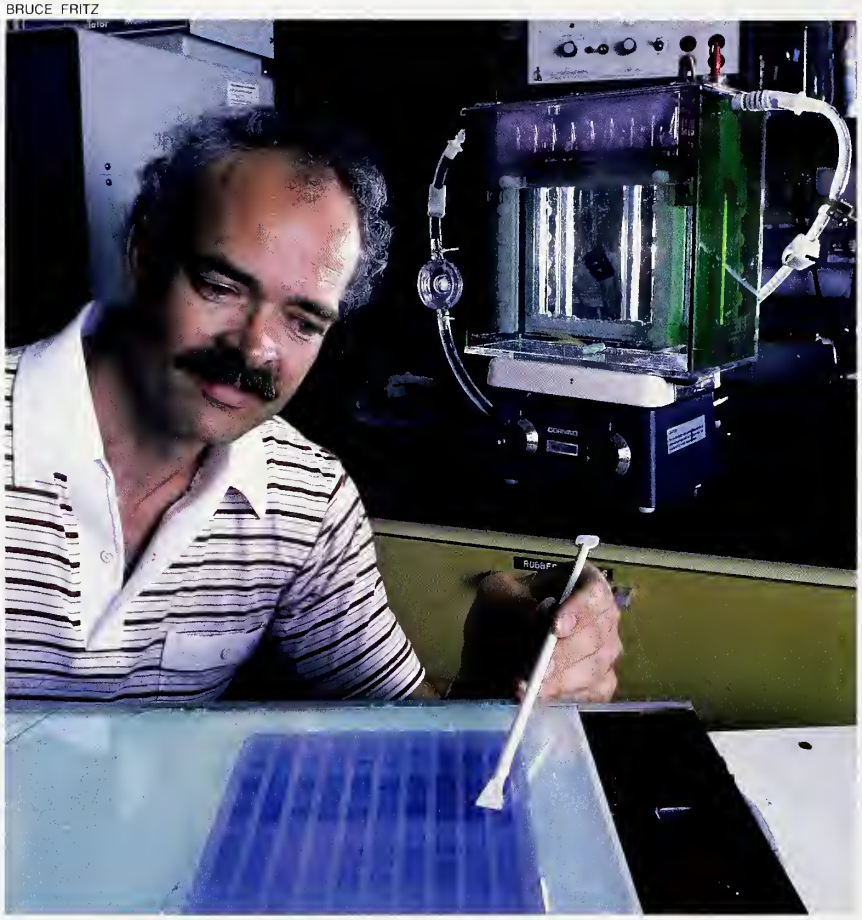
That makes new, high-protein wheats, such as NE86L177—an experimental line from ARS and University of Nebraska researchers—even more important. The line, which produces flours with high protein content and excellent breadmaking characteristics, will be shared with wheat breeders throughout the Great Plains.

Recently, wheat breeders used parts of a rye chromosome to enhance wheat yield and confer resistance to stem and leaf rusts, powdery mildews, and greenbugs. Today, 20 percent of the wheat acreage in the Great Plains is planted to wheat lines that carry the 1RS rye chromosome.

However, researchers now suspect that the chromosome may diminish quality. The chromosome carries genes that produce rye proteins known as secalins. "These proteins are biochemically different from the wheat proteins—the gliadins and glutenins," says Graybosch. "Secalins may account for the poor mixing and baking characteristics of flours from 1RS-carrying wheats."

If this is true, researchers may attempt to genetically engineer the rye chromosome to reduce secalin production in future wheats. But other factors, such as the amount or rate of nitrogen fertilizer applied, might also affect total protein quality. The Nebraska researchers are already investigating this possibility.

BRUCE FRITZ



Chemist George Lookhart of the U.S. Grain Marketing Research Laboratory, Manhattan, Kansas, identifies wheat varieties according to the unique patterns formed when proteins are translated electrophoretically into dark bands of varying intensity. (K-1783-4)

Probing the Puzzling Glutenins

Bakers have wheat's glutenins to thank for prized breadmaking qualities—like high loaf volume. That's why scientists are anxious to find out as much as possible about these important proteins.

A new biotechnological probe might help. The promising probe comprises antibodies that seek and bind to glutenins. It may prove a faster, easier way to accurately track and measure them, says ARS chemist Donald D. Kasarda at Albany, California.

To test the probe, he has joined chemist David L. Brandon, also at the Western Regional Research Center in Albany; Unna Stenram, a visiting scientist from Sweden, working at Albany; and plant physiologist Thomas W. Okita at Washington State University, Pullman. Okita was the first to discover that the probe reacts with wheat glutenins.

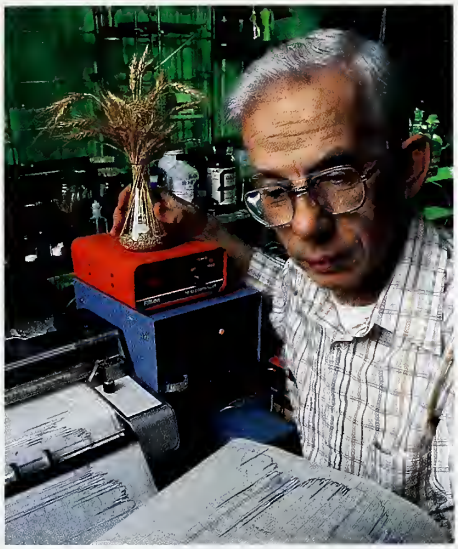
Complicating the glutenin studies is the fact that all glutenins aren't created equal. Some scientists speculate that glutenins with high molecular weights give dough added strength and elasticity and so make

better breads and rolls. Enhanced resilience enables doughs to remain strong throughout the tearing and crushing of kneading.

Kasarda and H. Peggy Tao at Albany are exploring the structure of glutenins of assorted weights, shapes, and sizes. Genetic engineering should make it possible to modify these natural proteins. That might yield superior glutenins capable of outperforming those in wheat today.

Glutenin's disulfide bonds, Kasarda says, might hold clues to engineering ideal proteins that will delight 21st-century bakers. These bonds can link one part of a glutenin chain to another part of that same chain. And perhaps even more important, the bonds make it possible for one glutenin protein to crosslink with another.

KEITH WELLER



Chemist Floyd Huebner separates proteins of hard red wheat using a high-performance liquid chromatograph. (K-3621-10)

Trouble is, no one knows which bonds, or how many of them, impart extra strength to high-glutenin flours. Discovering more about the vital bonds, says Kasarda, may push forward design of blueprints for tomorrow's super glutenins.

Sending Wheat to Market

When wheat goes to market, qualities such as kernel hardness or softness and test weight (how much flour a wheat is expected to yield) dictate the price that carloads of wheat will command.

Until recently, experienced grain inspectors could tell whether the kernels from a given variety of wheat

Cargo Surveys Scrutinize Exported Grain

Before cargo ships of U.S.-grown wheat set sail to foreign shores, federal inspectors scoop samples of grain to check its quality.

Some of the white wheat samples end up milled into flour that's mixed and baked into sugar snap cookies and other foods at ARS' Western Wheat Quality Laboratory in Pullman, Washington. Researchers bake these edibles not for snacks, but as food for science.

The ARS scientists grind out data on the baking qualities, protein content, and other characteristics of kernels and flour from white wheat grown in the Pacific Northwest.

The Pullman lab is one of three in the country performing quality tests for the U.S. Wheat Associates, an international marketing firm for American wheat growers. The association instituted the cargo survey in 1986 to assure foreign buyers that U.S. wheats will consistently meet their needs. The firm receives wheat samples from USDA's Federal Grain Inspection Service.

"The cargo survey is a service to the whole industry, from the farmer who grows the wheat all the way to the overseas customer who sits down to eat foods made with flour from our wheat," says Craig F. Morris, director of the Pullman Wheat Quality lab. His team's lab receives 1-kilogram samples (about 4 cups) from each of 165 cargos during the year.

Pullman scientists check flour yield and kernel protein and moisture levels. The researchers also assess the flour's merits when baked into Japanese spongecakes and sugar snap cookies or when shaped and boiled into Japanese udon noodles.

Between 85 and 90 percent of white wheat grown in northwestern states is exported to Japan and Korea or Middle Eastern countries such as Egypt and Pakistan. To date, the survey results, which are published in quarterly reports, have been remarkably consistent, according to Wheat Associates vice president James F. Frahm. As a safeguard, the firm plans to continue the survey indefinitely.

Morris, who oversees the testing in Pullman, says the uniformly high quality the researchers have seen so far "helps instill confidence in our foreign customers that they're getting a good product."—By **Julie Corliss,** ARS.

TIM McCABE

were hard or soft simply by looking at them. Their color and shape and tiny grooves on the surface were usually enough to go by.

But that's no longer the case. Hundreds of new wheat crosses enter national and international commodities markets every year. Crossbreeding of hard and soft wheats has produced new varieties that no longer conform to the traditional standards of either of these classes.

That has created an international marketing problem; a class designation is no longer a guarantee that the wheat will have the qualities the user needs. (Hard wheats make great bread and some types of pasta; soft wheats are best for cakes, pastries, quick breads, crackers, cookies, and snack foods.)

Analyzing wheat with near-infrared (NIR) radiation may be an answer, according to work by Karl Norris (now retired from ARS) and colleagues at the Instrument Sensing Laboratory, Beltsville, Maryland. Near-infrared wavelengths are those closest to visible light.

In view of the new wheat hardness studies from the Beltsville team, USDA's Federal Grain Inspection Service—responsible for wheat classification—now plans a pilot study on NIR's suitability for wheat hardness measuring. "We've already tested thousands of commercial wheat samples using NIR," says David Shipman, chief of the inspection service's Standards and Procedures Branch, "and found that NIR scores for a sample's average hardness were useful in distinguishing hard and soft varieties."

(Continued on page 18)

Engineers David Massie (left) and Karl Norris conduct tests with an early version of a grinding cylinder also used to determine hardness of wheat grains. (K-2333-1)

Corn Aids Wheat's Genetic Engineers

Unlike some crops, wheat and its fellow cereals—corn, rice, oat, barley, and rye—have proven extremely difficult to genetically engineer. Techniques for successfully slipping new genes into crops such as tomatoes or petunias typically don't work with the recalcitrant grains.

Now, however, a team of Albany, California, scientists has successfully inserted new genes into corn. Molecular biologist Michael E. Fromm, formerly with the ARS-University of California Plant Gene Expression Center, says the work may help researchers trying to coax new genes into other cereals, such as wheat.

In the corn experiments, cells took up genes fired from a gene gun. The device uses the force of .22-caliber cartridges to propel tungsten particles, coated with genes, into cells.

Scientists nurtured these cells into fertile plants that later passed new traits on to their offspring. The researchers used marker genes, that is, genes that are not useful to corn but help scientists test gene insertion. One marker gene, borrowed from fireflies, caused cells to glow faintly.

The clusters of corn cells that the scientists bombarded were developed by Charles L. Armstrong of Monsanto Company, St. Louis. Unfortunately, similar lab cultures apparently aren't yet available for wheat. So researchers like ARS chemist Frank C. Greene at Albany, who specializes in wheat, use corn cells in some experiments.

Gale F. Lorens, formerly at Albany, and Albany researchers Ann E. Blechl, Olin D. Anderson, and Greene use corn cells to test promoters—key portions of wheat genes. Promoters control gene activity. Some promoters, for example, are enhancers; they amplify gene action. The corn cells (technically known as protoplasts—cells stripped of walls) simplify the tedious task of finding out which promoter sections are the most powerful. Genetic engineers want to use the strongest promoters when they build new genes for drought resistance or other valuable traits.

In the laboratory, the Albany team showed that a wheat gene promoter, when hooked to a marker gene and then inserted into a corn protoplast, would go to work inside the protoplast. Normally, the wheat promoters act only in wheat.

Says Greene, "Now we can judge a promising wheat promoter in just a few days, instead of years."—By **Marcia Wood,** ARS.

JACK DYKINGA

New promise for genetically engineering wheat may come from recent experiments by Michael E. Fromm (right, now at Monsanto Company), ARS biological technician Rosalind E. Williams, and others who successfully transferred new genes into cells of another cereal crop corn using a gene gun. Cells yielded healthy, fertile parent plants that passed the new genes onto offspring, including the young, third-generation plant being examined here. (K-3701-7)

(Continued from page 16)

Shipman cautions, however, that NIR scores won't indicate whether a ground-up sample of thousands of kernels scooped from a truck or railroad car has hard and soft wheats mixed together. "Our wheat inspection system must have this capability as well," he says. ARS and the inspectors are working on new ways to meet this need.

Shipman expects NIR wheat hardness scores to serve as the primary reference for an improved wheat classification system. That's important because Congress has directed USDA to produce a system that's more objective and dependable.

Hint to Hardness: Flour Particle Sizes

Flour's particle sizes may be an alternative key to hardness. Flour milled from hard red spring wheat, for example, shows a different distribution of particle sizes than flour from hard red winter wheat, according to chemist Y. Victor Wu and chemical engineer Arthur Stringfellow (now retired) of ARS' Plant Protein Research unit, Peoria, Illinois.

The researchers used airstreams of different speeds to separate the flour particles by size. Jerold Bietz, in charge of the research, says the approach can be used to judge hardness of any wheat.

KEITH WELLER

Using airstreams of different speeds, chemical engineer Arthur Stringfellow (left) and chemist Y. Victor Wu separate flour particles by size. (K-3622-2)

Weight Standards Need Revamping

Another measure of wheat's quality is its test weight, or average weight per bushel. Grain merchants, milling companies, and others in the wheat business often use test weight to estimate how much flour they'll get from the wheat they buy. That estimate affects the price they'll pay the grower.

But today's test-weight standards are "too arbitrary, too unreliable, and too out of date" to be useful in predicting flour yield, says Patrick L. Finney. He directs the ARS Soft Wheat Quality Research unit at Wooster, Ohio.

Computerized analyses of 50 years of wheat quality records at Wooster indicate flour yield "relates to lots of factors," says Finney. "Low test weights are relevant only some of the time."

A full bushel of wheat might weigh as little as 51 pounds and still be more valuable to the miller than one with a test weight of 60 pounds, according to Finney. Conversely, the wheat could weigh more than its comparable worth.

Test weights presumably reveal whether the wheat was grown under conditions such as drought that could cause wheat to shrivel and lower its flour yield at the mill.

As it turns out, though, many growing conditions can bring about significant variations in test weight—without any shriveling or loss of milling value. Furthermore, because of their genetic backgrounds, test weights of many popular wheat varieties vary considerably even when they're grown under ideal conditions.

Among the eastern soft wheats alone, Finney says, average test weights of sound (full-flour value) wheat varieties ranged from 57 to

more than 62 pounds per bushel. "So farmers—or millers—won't get shortchanged during the grading process, test standards should accommodate all of the new varieties of wheat now coming to market," Finney says.

"That could make test weights far more useful, especially if the amount of sprouted wheat and broken and shrunken kernels were measured directly," he says. "Test weights, coupled with a record of the variety's typical range and a separate estimate of the number of damaged kernels, could then make a pretty good indicator of flour yield."

Guarding the Granary

Protecting wheat's quality once the crop leaves the field is a centuries-old problem still without the perfect solution. But new high-tech studies could someday give farmers, grain elevator operators, and warehouse managers a decided edge in their struggle to protect America's wheat harvest from insects that attack stored grain.

One innovative researcher has enlisted a female parasitic wasp called *Cephalonomia waterstoni*. When left to patrol bins of stored grain, the wasp will track down and parasitize larvae of the rusty grain beetle, a major pest of stored wheat and other grain. *C. waterstoni* zeros in on the beetle's unique chemical trail. In grain, the trail can persist for up to a week.

Ralph W. Howard, an ARS chemist, and Paul W. Flinn, a biologist, at the U.S. Grain Marketing Research Laboratory in Manhattan, Kansas, find the wasp's behavior interesting

BARRY FITZGERALD

At his soundproof studio in Gainesville, Florida, engineer J.C.Webb and lab technician Betty Weaver listen to sounds made by the lesser grain borer inside a grain of wheat. (K-1677-4)

JACK DYKINGA

While geneticist William Inwood (background) extracts protein from the endosperm tissue of wheat, chemist John Bernardin examines banding patterns of wheat proteins exposed to various temperatures. (K-3696-5)

Sun Thwarts Key Wheat Genes

The sun might be to blame if some of today's wheats end up unexpectedly low in glutenins, the proteins key to breadmaking quality.

ARS researchers John E. Bernardin and William B. Inwood at Albany have shown that summer temperatures of 99°F or more—common in many wheat-growing states—cause glutenin genes to stop working.

But genes that control another class of grain proteins—the gliadins—stay on the job until the thermometer hits 113°F. Wheats then end up with a higher gliadins-to-glutenins ratio. That's exactly the opposite of what seems best for breadmaking.

So that heat won't stymie glutenin production in wheats of the future, the two scientists are building an improved gene. They're attempting to splice a gliadin gene's on-off switch to glutenin-producing genes. Wheats containing the new gene should pump out glutenins even in 99°F weather, and perhaps until the temperature reaches gliadin's upper limit of 113°F, the scientists say.

To build that gene and get it accepted into healthy, fertile wheat plants' genetic makeup may take anywhere from 6 months to 4 or 5 years.—By **Marcia Wood,** ARS.

and potentially useful for biological control of the beetle. The next challenge: Find out how to keep the wasps on the job for longer periods.

At the same lab, entomologist David W. Hagstrum has discovered that insect damage to grain is not as silent an occurrence as one might expect. Through microphones positioned in the grain, the lesser grain borer has proven itself to be a noisy intruder, emitting sharp sounds that resemble popcorn popping.

"Those popcorn sounds we're hearing are the insects feeding inside kernels of grain," Hagstrum says. "The sound is inaudible to the human ear, but our acoustic system amplifies it up to 75,000 times."

The system is still being refined, but he says it will eventually provide a quick and easy method of detecting insects without removing grain samples from the bin. It should also help grain elevator operators estimate how many insects are present and where they are.

Conventional methods of detecting insects in grain include sifting grain samples, running samples through an x-ray machine, and measuring insect-produced carbon dioxide. But all three methods are more costly or less reliable than the acoustic system, which also has the added advantage of automated monitoring. Hagstrum says the system may be able to monitor several grain bins from a single central location and do so automatically.

Agricultural engineer J.C. Webb and entomologist Kenneth W. Vick, of the agency's Insect Attractants, Behavior, and Basic Biology Research Laboratory, Gainesville, Florida, developed experimental sound detection methods for insects in the early 1980's and are cooperators in this research.

ROB FLYNN

In their simulated warehouse at Savannah, Georgia, research entomologists Henry Highland (standing) and Michael Mullen survey insect damage to test packages of cereal-based foods. (K-3672-8)

Insect-Safe Packaging

Meanwhile, ARS scientists in Savannah, Georgia, are working on ways to ensure that insects don't crawl into your wheat flakes after they are packed and on their way to the supermarket.

Scientists in the Environmental and Special Problems Research unit study various ways insects enter packaged wheat products such as cereal, flour, cake mixes, and pet foods. Entomologist Henry A. Highland says insects have an uncanny ability to find the slightest nook, cranny, or opening in a seemingly intact package.

"Once they get in, they set up housekeeping," Highland says. "Insects particularly like products with a high protein content."

Insects generally find open seals or bore through packages. Because products are packaged at high speeds, Highland says, it's pretty hard to make all packages insect-tight.

His job is to find weaknesses in packaging and study how insects enter products. This is done by filling a typical package with a product and then fumigating it, to make sure it's free of live insects at the start of the experiment.

The experimental packages are placed in small warehouses that contain at least 16 different species of postharvest insects, Highland says. The packages are stored and tested for 6 months—enough time to produce three generations of insects.

Results indicating how many insect species infest packaged products are shared with industry. "We want to provide information so food processors can design and produce packages that will better resist insects," Highland says. "By maintaining product quality, good packaging will help in our exports."—By **Matt Bosisio, Linda Cooke, Ben Hardin, Bruce Kinzel, Steve Miller,** and **Marcia Wood,** ARS. ◆

Lethal Genes May Wipe Out Unwanted Pollen

Hardy vines that yield plump, juicy tomatoes—the pride of America's backyard gardeners—often start life overseas, nurtured by field workers in countries such as Taiwan.

These laborers are matchmakers to the plants they tend and midwives to the elite female plants that produce high-performance hybrid seed—the kind that ends up in little packets you can buy at your local nursery.

But scientists such as ARS' Sheila M. McCormick at Albany, California, want to give seed companies new, more efficient, and more economical ways to produce world-class hybrid seed, using tools of modern biotechnology. Not only tomatoes, but dozens of other crops could benefit, says McCormick, who is at the Agricultural Research Service/ University of California Plant Gene Expression Center.

Today, to produce superior hybrid seed, field workers first pull anthers (the male, pollen-producing parts) off certain plants. This readies those plants for their new role as the female, seed-yielding parents. And it ensures that the altered plants can't fertilize themselves with their own pollen, as they might otherwise do in nature. (Tomatoes, like most other crops, produce both male and female parts on each plant.)

Later, the workers use pollen from unaltered plants, often growing in adjacent rows, to pollinate the female plants. Sometimes the wind will do this job for them.

Once pollinated, the female plants form hybrid seed that's destined for the commercial grower or the backyard gardener.

This careful crossbreeding of one superior parent plant with another produces hybrid seed that typically boasts the best features of both relatives—reliable yields, resistance to disease, and an attractive color. And hybrids are usually robust, thanks to a highly prized characteristic known as "hybrid vigor."

McCormick is working on a futuristic scheme that would drastically simplify production of superior hybrid seed and cut costs. The idea: Use genetic engineering to create plants with pollen that self-destructs when the breeder wants it to. That strategy requires giving the plant a lethal gene that the breeder could somehow turn on or off at will. "For this to work," says McCormick, "the gene has to be pollen-specific, that is, it has to attack pollen without damaging other parts of the plant."

JACK DYKINGA

In studies to reduce costs and simplify production of superior hybrid seed, geneticists Sheila McCormick and David Twell examine a DNA-sequence gel of the LAT52 gene promoter. (K-3531-3)

McCormick and colleagues David Twell and Judy K. Yamaguchi have already pinpointed two different pieces of genetic material that work almost exclusively in pollen and might be ideal for building the artificial gene.

The newly identified gene segments, called LAT52 and LAT59, are "promoters" that act like a light switch, turning genes on and off. The team is apparently among the first to identify, in flowering plants, promoters that are "essentially pollen-specific," says McCormick.

Such promoters could be hooked to a gene that's toxic to pollen yet harmless to other plant parts, or to us. Although both promoters seem promising for that job, LAT52 restricts itself more to pollen while LAT59 is "slightly leaky." The scientists detected it in roots as well as in pollen. "We don't know yet if this leakiness will hurt the roots," she says.

The team tracked promoters by first linking them to tracer-type genes and then inserting them into tomato cells.

The scientists nurtured the cells to form plants. When the plants started to flower, the scientists checked pollen grains, as well as roots, stems, leaves, petals, and other plant parts, looking for the tracer genes.

In some experiments, McCormick and coworkers linked a tracer gene called GUS (short for beta-glucuronidase) to the LAT52 or LAT59 promoter. To test the promoters, they exposed parts of the maturing tomato plant to a compound that turns samples dark blue if GUS is active.

Pollen grains came out a deep blue, but other plant parts didn't. "That's proof the promoter works in pollen yet is inactive elsewhere," says McCormick.

A device called a gene gun has speeded their search for promising promoters that could turn on killer genes in pollen. The gun propels genetic material—such as the GUS gene or other tracer genes and their promoters—into pollen grains. About 6 hours after bombarding the pollen, we can check to see whether

The idea: Use genetic engineering to create plants with pollen that self-destructs when the breeder wants it to.

the GUS gene is 'on'— instead of having to wait 4 to 6 months for laboratory-reared plants to produce pollen," she explains.

Once an ideal promoter is ready to use, the choice of which toxic gene to hook onto it will be left up to seed companies, who may already have candidates in mind. "Our job is to find out if this strategy for killing pollen will work," says McCormick.

The potentially lethal gene the Albany team chose for study is *tms2*, borrowed from a bacterium, *Agrobacterium tumefaciens*.

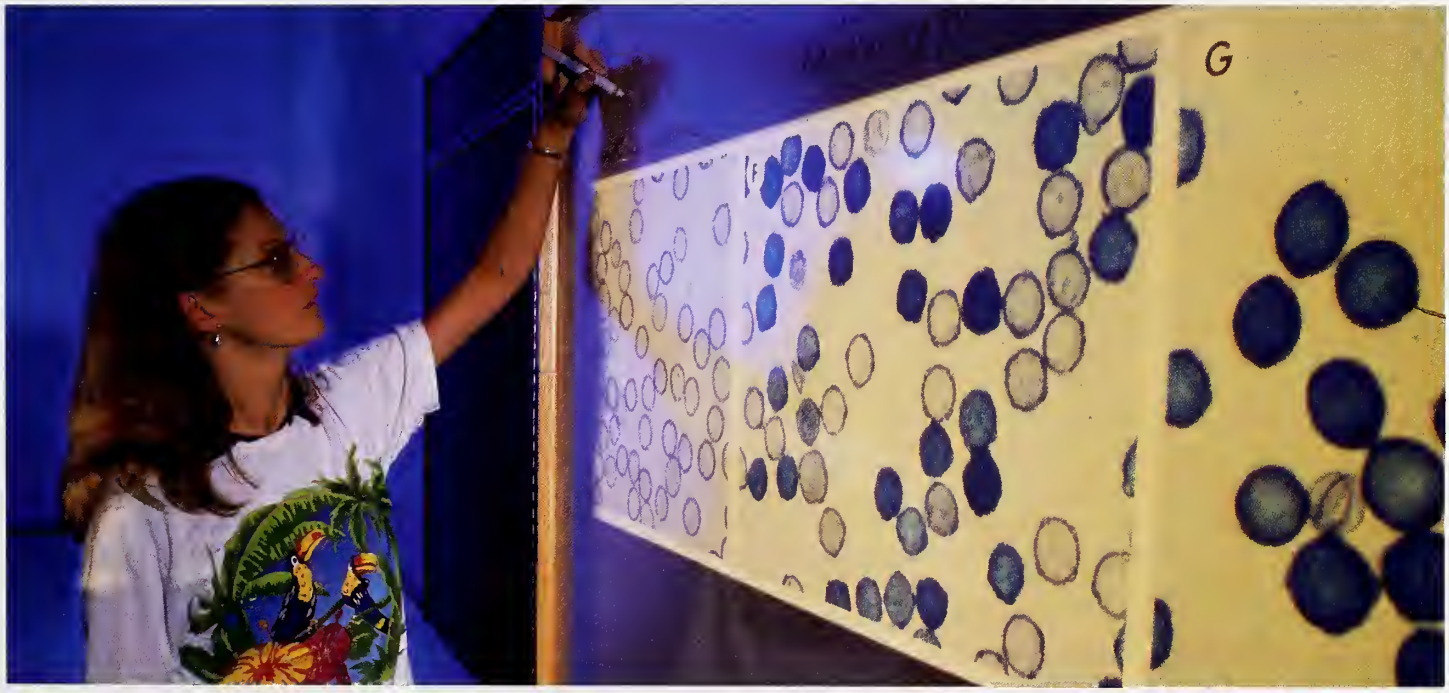
This gene should give pollen the ability to convert a compound called IAM, (indole acetamide) into a plant hormone, IAA (indole acetic acid). "Plants don't normally contain IAM," explains McCormick. "But if you sprayed one with IAM, we think the new gene in pollen would likely respond by processing it into IAA. Other plant parts don't have this gene, so they shouldn't be affected."

If IAA is a normal component of healthy plants, how could it endanger pollen? "The new gene might force pollen to overproduce the hormone, so that there would be a toxic amount in pollen. After all, anything—even something such as salt—can be lethal if you get too much of it."

McCormick estimates that sometime this year—perhaps as early as this summer—she'll know if this scheme to selectively kill pollen will work. If it does, some of tomorrow's pollen will be doomed. But other grains of pollen may yield exciting new hybrids to please growers and gardeners alike.—By **Marcia Wood,** ARS.

Sheila M. McCormick, David Twell, and Judy K. Yamaguchi are with the USDA ARS/University of California Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710 (415) 559-5906. ◆

JACK DYKINGA



Sheila McCormick reviews a projection of tobacco pollen from transgenic plants carrying the LAT52-GUS gene. (K-3531-10)

Fish Oil Can Have Its Drawbacks

According to a growing body of evidence, fish oil would appear to be the elixir of the late 20th century. Studies have shown it can ease the itching and scaling of psoriasis, dampen the pain of migraine headache and rheumatoid arthritis, and help prevent coronary artery disease by reducing blood clotting and the level of fat that circulates in the blood.

But the Food and Drug Administration is not convinced of the oil's efficacy and has recently disallowed all health claims for the capsules. Fish oil is considered a food, says FDA spokesperson William Grigg. The agency rarely allows health claims on foods unless the evidence is overwhelming. "And we don't know the long range effects," Grigg says.

There's good reason for caution, according to researchers at the ARS Human Nutrition Research Center on Aging at Tufts University. In the rush to acknowledge the positive effects of fish oil, some of the negatives have been overlooked.

"Feeding fish oil has been a classic method for inducing vitamin E deficiency in experimental animals," says Mohsen Meydani, who specializes in the toxic effects of nutrients at the Human Nutrition Research Center on Aging at Tufts.

That's because fish oil is more vulnerable to oxidation than most other fats so more of the antioxidant vitamin is required for protection. Without it, oxidized fats themselves

become oxidizing agents setting off a chain reaction. We've all experienced the rapid spoilage and putrid smell of dead fish.

In living animals and people, unchecked lipid oxidation can damage a host of critical molecules inside cells, including DNA.

Manufacturers of fish oil capsules add synthetic antioxidants and vitamin E to preserve the oil's life on the shelf. But is enough added to preserve its life in the human body? The fish oil we eat becomes an integral part of cell membranes and other structures. Without enough vitamin E, these structures may be oxidized and their function impaired. What's more, Meydani had earlier found that rats fed fish oil absorbed significantly less vitamin E than the animals fed either corn or olive oil.

He says his recent study of 35 women suggests that the level of vitamin E in most fish oil preparations on the market today "may not be enough to maintain a balance between the highly unsaturated fats in fish oil and vitamin E levels in the human blood."

He analyzed monthly blood samples from the women for vitamin E and oxidized fats, known as lipid peroxides, as they took 3 grams of fish oil daily for 3 months. The supplements contained a total of 6 international units (IU) of vitamin E, or 60 percent of the RDA for women. Since 15 of the women were in their mid-twenties and 10 were in their early sixties, the results show how concentrated fish oil affects both young and older people.

After 3 months of supplementing, blood levels of vitamin E did not change significantly. But levels of triglycerides dropped, giving the women a higher ratio of the vitamin to the fats they are supposed to protect. It apparently wasn't enough to keep the fragile fats from being oxidized, says Meydani, especially in the older women.

Damaging lipid peroxides increased an average of 30 percent in the young women and 50 percent in the older group. Half the young women did not have any increase, but all of the older women did.

The results support other evidence that long-term intake of fish oil capsules increases the need for vitamin E, he says. "People, older people in particular, may have to take more tocopherol (vitamin E) than what is in the capsules," especially if they smoke, take medications that become oxidizers in the body, or live in areas high in atmospheric ozone.

Fish oil capsules seem to be effective in relieving some inflammatory diseases...But the fish oil supplement also depresses the immune system's response to simulated infection.

According to Meydani, one pharmaceutical company scientist has suggested that the amount of vitamin E in fish oil capsules be doubled or tripled. Meydani has analyzed most of the fish oil preparations on the market today and found more than a three-fold difference in vitamin E levels. The capsules used in this study scored in the middle of the range for commercially available fish-oil capsules, a range that varied from 0.02 to 2.24 mg per gram.

Simin Nikbin Meydani, an immunologist at the Center, has another suggestion based on changes in the women's immune response: "Maybe we're giving too much fish oil. Perhaps we could find a level where you can see the benefits without the reduction in immunity."

She says taking fish oil capsules seems to be effective in relieving some inflammatory diseases: It significantly reduced immune system signals—certain cytokines—that are primarily involved in inflammation. And the effect was far more pronounced in the older women, she says, as much as eight times greater for one cytokine.

But the fish oil supplements also depressed the immune system's response to simulated infection. Again the effects were significant and more dramatic in the older women. The supplements reduced by 63 percent their production of a cytokine that is primarily involved in increasing the number of T cells and other lymphocytes against foreign invaders. And it reduced by 36 percent the ability of T cells to divide and conquer.

Since these immune functions normally decline with age, says Simin Meydani, "the beneficial anti-inflammatory effect of fish oil must be weighed against its effect on T-cell-mediated immune response, especially in older women."

So, while fish oil may be an elixir for some ailments, it should be used with care and with awareness of possible side effects.—By **Judy McBride, ARS.**

Mohsen and Simin Meydani are at the USDA Human Nutrition Research Center on Aging at Tufts, 711 Washington St., Boston, MA 02111 (617) 556-3126/3129. ◆

Stomach Worm Interferes With Vaccine

A microscopic worm may be creating costly and dangerous holes in cattle producers' to vaccinate their herds.

The worm, *Ostertagia ostertagi*, is nothing exotic. Also known as the medium brown stomach worm, it is the most common economically important gastrointestinal parasite of cattle in the world's temperate regions.

But aside from the intestinal havoc it wreaks, *O. ostertagi* may also reduce the effectiveness of initial calfhood vaccinations against ailments such as brucellosis and blackleg.

"Our research has shown that the parasite produces a substance that interferes with the ability of the host animal's cells to make antibodies," says ARS microbiologist Phillip H. Klesius.

"We also know this immunosuppressant substance interferes with the ability of the lymphocytes—white blood cells—to recognize the substance and proliferate in response to it. It's not that the parasite is destroying the animal's immune cells; it just doesn't allow them to work as effectively."

Klesius is research leader at ARS' Animal Parasite Research unit at Auburn, Alabama. Studies are under way there in cooperation with MSD-AGVET, the animal health branch of Merck Pharmaceuticals of Rahway, N.J., to learn whether the parasite substance is interfering with vaccinations. The ARS research team includes, in addition to Klesius, veterinary medical officers Daniel E. Snyder and Debra A. Cross.

"We've infected calves with *Ostertagia ostertagi* and studied their immune response," Klesius says. "We're showing that primary immunity derived from calfhood vaccinations is impaired by the parasite, but booster immunization is not.

"When your calf gets the second shot, that boosts the activity of its immune system enough to overcome the parasite's substance. But booster shots are not always given."

Those cattle producers who skip booster shots could be running a risk, Klesius warns: "If your calves are continually exposed to *Ostertagia* at a young age and then you try to immunize them, their immune responses may be less vigorous than in animals kept free of the parasite. Your animals could still get sick from the diseases you're vaccinating against."

The parasite life cycle begins when an *Ostertagia*-infected animal sheds the parasite eggs in its feces. These eggs develop and hatch and eventually produce infective larvae that make their way onto the grass. As the cattle graze, the larvae are picked up. They further develop and become adults in the cow's abomasum, or true stomach. There they interfere with digestion of protein.

"Gradually, as the animal gets older, it builds up resistance to the parasite," says Klesius. "It can still get infected, but the parasites won't cause as much damage to the stomach."

However, in cattle less than 2 years old, the parasite hits hard. More pounds of feed are required to produce a pound of meat on the animal, weight gains are lower, and the animals mature more slowly—problems that may cost the farmer up to 10 cents a pound in profits, Klesius says.

"If we can identify exactly how this parasite substance is working, we may be able to produce an antibody against it," he says. "Then we could just give the calves a vaccination against the parasite after they're weaned."—By **Sandy Miller Hays**, ARS.

Phillip H. Klesius is in USDA-ARS Animal Parasite Research, P.O. Box 952, Auburn, AL 36831-0952 (205) 887-3741. ◆

Higher Protein, Great Yield

An ARS scientist believes he has broken the deadlock between high yield and high protein content in soybeans.

Plant geneticist Joseph W. Burton is on the verge of having a soybean variety with a 3.4-percent increase in protein that also has excellent yield.

This increase, from about 42 percent to almost 46 percent, translates to a possible $1.00 to $1.50 per bushel in extra revenue to the farmer, according to Burton, who is based at the ARS Soybean and Nitrogen Fixation Research unit in Raleigh, North Carolina.

"Currently, there is no surplus of soybean meal (protein). The market can use all the protein we can produce from soybeans, so there is a real need for this kind of a new soybean variety," Burton says.

In the past, soybeans that had high levels of protein also had lower yielding ability.

"With higher protein, yields always dropped off to the point that there was little if any net gain in total protein," explains Burton.

In conventional breeding programs, the two traits—protein and yield—have acted as if they were coupled or linked genetically, he says.

To decouple the traits, Burton used a breeding process called restricted index selection, a method employed most often in animal breeding.

Burton began by mating high-yielding soybeans with high protein types. These matings resulted in a very genetically variable population of offspring with an average protein content of 45.6 percent.

Families from this population were tested independently for each of the two traits: yielding ability and protein level. Those data were then used to calculate the restricted selection index, which statistically related the range of protein level and potential for yield.

The top 20 percent, based on their index values, were intermated to begin another selection cycle. Four selection cycles have been completed.

"The advantage of this system is that it maintains a lot of genetic variation—a lot of diversity—for yielding ability in the offspring and maintains protein at a constant level," Burton says. "Selected offspring are then used in more a conventional pedigree breeding method to develop a pure line or variety as quickly as possible."

It took about 10 years of breeding, but Burton now believes he is only a few years away from releasing the first high-yielding soybean with the higher protein level. The results of final testing will determine whether the

soybean is released as improved germplasm or as a new variety. "But we'll have something out in a year's time," Burton says.—By **J. Kim Kaplan,** ARS.

Joseph W. Burton is at the USDA-ARS Soybean and Nitrogen Fixation Research Unit, North Carolina State University, Box 7620, Raleigh NC 27695 (919) 737-3171. ◆

Corn Mold Hazard

In some areas of South Africa and China where people grow and eat their own corn, there is a high rate of esophageal cancer. In the United States, horses are dying from a disease that destroys their brains. A possible connection: moldy corn.

Leucoencephalomalacia (ELEM) is a fatal disease in horses often associated with eating moldy corn. Outbreaks have been reported as far back as the early 1900's, and last year "numerous reports of horse deaths from ELEM were made in the corn-producing areas of the United States," says Ron D. Plattner, a chemist at ARS' Northern Regional Research Center in Peoria, Illinois.

The corn is infected with the fungus *Fusarium moniliforme*. South African researchers have shown the fungus was related to the horse disease and isolated toxins from the fungus called fumonisins. ARS researchers took their work one step further by showing that the fumonisins were present in corn samples that killed horses.

"Because *F. moniliforme* is more often found in corn than *Aspergillus flavus*, the mold that causes aflatoxin, it may be even more significant than aflatoxin if its link to animal or human diseases is proven," says Plattner.

A. flavus isn't always present in corn, but *F. moniliforme* is—sometimes causing problems and sometimes not. Stalk and ear rot are two corn diseases. But the question is open regarding what, if any, role fumonisins play in animal diseases.

In Athens, Georgia, ARS supervisory pharmacologist William P. Norred says besides being implicated in the horse disease, fumonisins have been linked with liver cancer in rats.

Athens scientists are beginning to look at biochemical responses in rats fed the toxins. But the main obstacle has been producing enough toxins.

That obstacle has now been removed by Plattner and coworkers in Peoria, who have isolated and measured toxins produced by the fungus. They have also developed standards and analytical methods to test corn for fumonisins.

"We can produce enough fumonisins to supply analytical standards to other ARS laboratories or veterinarians interested in testing feed before it's fed to horses or other livestock," he says.

Using rats as a model, researchers at Athens will look at organs and tissues the toxin affects and at what dose level. They will also check for traces of toxin residues in meat, poultry, eggs, and milk. "For now we're gathering data to see if fumonisins are really responsible for a variety of animal diseases," Norred says.— By **Linda Cooke** and **Bruce Kinzel,** ARS.

William P. Norred is at the USDA-ARS Toxicology and Mycotoxin Research Unit, P.O. Box 5677, Athens, GA 30613 (404) 546-3158.Ron D. Plattner is at the USDA-ARS, Northern Regional Research Center, 1815 N. University St., Peoria, IL 61604 (309) 685-4011.◆

Prolactin: Part of Milk's Riddle

"We have known for many years that prolactin, a pituitary hormone, starts a cow's milk production at the end of pregnancy. But there are a number of questions about its function and how it works," says Anthony V. Capuco, an animal physiologist with the Agricultural Research Service in Beltsville, Maryland.

"We know that prolactin prepares the cows' mammary glands to produce milk by binding to and changing the shape of a protein molecule or receptor on the surface of the gland's cells. This starts a series of events that results in lactation," says Capuco.

Knowing how prolactin binds to this "biological switch" and turns it on to stimulate mammary gland cells to produce milk will help us understand how cows and other mammals regulate milk production.

Doctoral student James J. Smith, who is working on this as part of his doctoral thesis, reports, "Our new understanding may lead to methods that would allow farmers of the future to "program" a pregnant cow to produce larger amounts of milk without increasing feed input—lower producers could be improved."

Capuco and Smith found that while mammary tissue of laboratory mammals such as mice and rabbits have both high and low molecular weight receptors for prolactin, mammary glands of cattle lack the high molecular weight form of the prolactin receptor.

"Cows are unique in that they produce relatively large amounts of milk and they don't get mammary tumors. Prolactin has been implicated in certain types of mammary tumors," says Capuco.

Does the lack of receptor sites for the high molecular weight receptor protect cows from prolactin-related mammary tumors? "At this point we can only speculate, but the question is intriguing, especially if this knowledge could lead to prevention of mammary tumors in humans," says Capuco.

"We have found that cows process the prolactin receptor differently during pregnancy than at other times. This may provide important clues to how prolactin works," says Smith.

In pregnant dairy cattle, blood prolactin increases a short time before a calf is born and initiates the process of milk production. Once the cow's mammary glands start producing milk, prolactin no longer seems to be very important for milk production. In contrast, humans and many other mammals require it throughout lactation.

Says Capuco: "We hope to shed some light on the apparent differences in how humans, rats, and cattle use prolactin to regulate production of milk."—By **Vince Mazzola,** ARS.

Anthony V. Capuco and James J. Smith are with the USDA-ARS Milk Secretion and Mastitis Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705 (301) 344-1672. ◆

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